

What is claimed is:

1. An isolated nucleic acid molecule encoding a polypeptide which has 2,5-DKG permease activity.

2. The isolated nucleic acid molecule of claim 1,
5 comprising a nucleotide sequence having at least 40% identity to a nucleotide sequence selected from the group consisting of SEQ ID NOS:1, 3, 5, 7, 9 and 11.

3. The isolated nucleic acid molecule of claim 1,
10 comprising a nucleotide sequence having at least 80% identity to a nucleotide sequence selected from the group consisting of SEQ ID NOS:1, 3, 5, 7, 9 and 11.

4. The isolated nucleic acid molecule of claim 1,
comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS:1, 3, 5, 7, 9 and 11.

15 5. The isolated nucleic acid molecule of claim 1, comprising a nucleotide sequence which encodes a polypeptide having at least 40% identity to an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 6, 8, 10 and 12.

20 6. The isolated nucleic acid molecule of claim 1, comprising a nucleotide sequence which encodes a polypeptide having at least 80% identity to an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 6, 8, 10 and 12.

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7. The isolated nucleic acid molecule of claim 1, which encodes a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOS: 2, 4, 6, 8, 10 and 12.

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8. The isolated nucleic acid molecule of claim 1, which comprises a nucleotide sequence encoding a peptide having at least 10 contiguous amino acids of any of SEQ ID NOS:2, 4, 6, 8, 10 and 12.

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9. The isolated nucleic acid molecule of claim 1, which comprises a nucleotide sequence encoding a peptide having at least 10 contiguous amino acids of at least any two of SEQ ID NOS:4, 8 and 12.

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10. The isolated nucleic acid molecule of claim 1, which comprises a nucleotide sequence encoding a peptide having at least 10 contiguous amino acids of at least any two of SEQ ID NOS:2, 6 and 10.

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11. The isolated nucleic acid molecule of claim 1 operatively linked to a promoter of gene expression.

12. The isolated nucleic acid molecule of claim 11, wherein said promoter is a *lac* promoter.

13. A vector comprising the isolated nucleic acid molecule of claim 11.

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14. The vector of claim 13, comprising a spectinomycin resistance gene.

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15. A bacterial cell, comprising the vector of claim 13.

16. The bacterial cell of claim 15, wherein said isolated nucleic acid molecule comprises a nucleotide
5 sequence which encodes a polypeptide having an amino acid sequence at least 80% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:2, 4, 6, 8, 10 and 12.

17. The bacterial cell of claim 16, wherein said
10 amino acid sequence is at least 95% identical to SEQ ID NO:8.

18. The bacterial cell of claim 17, further comprising an isolated nucleic acid molecule comprising a nucleotide sequence which encodes a polypeptide having an
15 amino acid sequence at least 95% identical to SEQ ID NO:4.

19. The bacterial cell of claim 17, further comprising an isolated nucleic acid molecule comprising a nucleotide sequence which encodes a polypeptide having an amino acid sequence at least 95% identical to SEQ ID NO:10.

20. The bacterial cell of claim 15, which is of the genus *Klebsiella*.

21. The bacterial cell of claim 15, which is deficient in endogenous 2,5-DKG activity.

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22. The bacterial cell of claim 21, comprising an isolated nucleic acid molecule encoding a polypeptide having at least 80% identity to SEQ ID NO:14 and 2-keto reductase activity.

5 23. The bacterial cell of claim 21, comprising an isolated nucleic acid molecule encoding a polypeptide having at least 80% identity to SEQ ID NO:16 and 5-keto reductase activity.

10 24. The bacterial cell of claim 15, which is of the genus *Pantoea*.

25. The bacterial cell of claim 15, which expresses an enzyme that catalyzes the conversion of 2,5-DKG to 2-KLG.

15 26. The bacterial cell of claim 25, which expresses enzymes that catalyze the conversion of glucose to 2,5-DKG.

27. The bacterial cell of claim 26, which is deficient in endogenase 2-keto-reductase activity.

20 28. An isolated oligonucleotide, comprising at least 20 contiguous nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID NOS:1, 3, 5, 7 and 9.

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29. The isolated oligonucleotide of claim 28, comprising at least 50 contiguous nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID NOS:1, 3, 5, 7 and 9.

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30. An isolated oligonucleotide, comprising a nucleotide sequence encoding a peptide having at least 10 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 6, 8 and 10.

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31. A method of making the isolated nucleic acid molecule of claim 1, comprising introducing into a bacterial cell deficient in endogenous 2,5-DKG permease activity one or more expressible nucleic acid molecules, identifying a cell having 2,5-DKG permease activity following said introduction, and isolating the introduced nucleic acid molecule from said cell.

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32. The method of claim 31, wherein said one or more isolated nucleic acid molecules is a genomic DNA library.

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33. The method of claim 32, wherein said genomic DNA library is prepared from an environmental sample.

34. The method of claim 31, wherein said bacterial cell is a *Klebsiella oxytoca* deficient in *yiaX2*.

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35. The method of claim 31, wherein said bacterial cell comprises an isolated nucleic acid molecule encoding a polypeptide having at least 80% identity to SEQ ID NO:14 and 2-keto reductase activity, and a polypeptide
5 having at least 80% identity to SEQ ID NO:16 and 5-keto reductase activity.

36. A method of using the isolated nucleic acid molecule of claim 1 to enhance 2-KLG production, comprising expressing the polypeptide encoded by said nucleic acid
10 molecule in a bacterial which expresses an enzyme that catalyzes the conversion of 2,5-DKG to 2-KLG.

37. The method of claim 36, wherein said bacterial cell further expresses enzymes that catalyze the conversion of glucose to 2,5-DKG.

15 38. The method of claim 37, wherein said bacterial cell is deficient in endogenous 2-keto reductase activity.

39. The method of claim 36, wherein said
20 bacterial cell is of the genus *Pantoea*.

40. The method of claim 36, further comprising converting said 2-KLG to ascorbic acid.

41. An isolated polypeptide which has 2,5-DKG permease activity.

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42. The isolated polypeptide of claim 41, comprising an amino acid sequence having at least 40% identity to an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 6, 8, 10 and 12.

5 43. The isolated polypeptide of claim 41, comprising an amino acid sequence having at least 80% identity to an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 6, 8, 10 and 12.

10 44. The isolated polypeptide of claim 41, comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 6, 8, 10 and 12.

45. The isolated polypeptide of claim 41, comprising at least 10 contiguous amino acids of any of SEQ ID NOS:2, 4, 6, 8, 10 and 12.

15 46. An isolated peptide, comprising at least 10 contiguous amino acids of any of SEQ ID NOS:2, 4, 6, 8 and 10, wherein said peptide is immunogenic.

47. An antibody specific for the isolated polypeptide of claim 44.

20 48. An antibody specific for the isolated peptide of claim 46.

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